Journal of Plant Physiology **164** (2007) 536—543

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JOURNAL OF PLANT PHYSIOLOGY

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Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*)

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Received 5 December 2005; accepted 23 February 2006

KEYWORDS
Antioxidant;
Iron deficiency;
Maize;
Nitric oxide;
Oxidative damage

Summary

The effects of nitric oxide (NO) in protecting maize (*Zea mays*) leaves against iron deficiency-induced oxidative stress were investigated. The increased contents of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{-\bullet}$) due to iron deficiency suggested oxidative stress. The increased contents of thiobarbituric acid-reacting substances (TBARS) and the decreased contents of protein-bound thiol (PT) and non-protein-bound thiol (NPT) indicated iron deficiency-induced oxidative damage on proteins and lipids. Sodium nitroprusside (SNP), a nitric oxide (NO) donor, partially reversed iron deficiency-induced retardation of plant growth as well as chlorosis. Reduced contents of H_2O_2, $O_2^{-\bullet}$, TBARS and increased contents of PT and NPT also indicated that NO alleviated iron deficiency-induced oxidative damage. The activities of SOD and GR decreased sharply while the activities of CAT, POD and APX increased under SNP treatment. Our data suggest that NO can protect maize plants from iron deficiency-induced oxidative stress by reacting with ROS directly or by changing activities of ROS-scavenging enzymes.

Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate; CAT, catalase; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GR, glutathione reductase; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; NBT, nitroblue tetrazolium; NO, nitric oxide; NPT, non-protein-bound thiol; $O_2^{-\bullet}$, superoxide radical; POD, peroxidase; PT, protein-bound thiol; PVP, polyvinylpolypyrrolidone; ROS, reactive oxygen species; SNP, sodium nitroprusside; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reacting substances

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doi:10.1016/j.jplph.2006.02.011

Introduction

Plants are prone to produce excessive reactive oxygen species (ROS) under adverse conditions such as drought, extreme temperature, high irradiance, atmospheric pollution, pathogen attack, etc. ROS includes singlet oxygen (O_2^1), superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). They are considered to be toxic byproducts of aerobic metabolism because they can react with various cellular components to induce oxidative damage (Mittler, 2002). ROS detoxification is carried out by a network of reactions involving enzymes such as superoxide dismutase (SOD: EC 1.15.1.1), catalase (CAT: EC 1.11.1.6), peroxidase (POD: EC 1.11.1.7), ascorbate peroxidase (APX: EC 1.11.1.11), NADPH-dependent GSSG reductase (GR: EC 1.6.4.2) and low-molecular-weight antioxidants such as ascorbate (ASC), glutathione (GSH), carotinoids, α-tocopherol and flavonoids (Mittler, 2002).

Iron, because of its binding to heme groups, iron-sulfur clusters or direct association with proteins, is recognized as a bioactive element, which is essential for many cellular functions (Guerinot and Yi, 1994). Although abundant in crust, iron availability is always limited especially in calcareous soil (high pH). Iron deficiency impairs chlorophyll biosynthesis and chloroplast development in both dicotyledonous and monocotyledonous species (Graziano et al., 2002) and reduces crop the primary productivity.

As an abiotic stress for plants, iron deficiency was shown to affect the expression and the activity of certain peroxidase isoenzymes and induces secondary oxidative stress in dicotyledonous species (Ranieri et al., 2001). Recently, Zaharieva et al. (2004) found in sugar beet roots that iron deficiency resulted in the decreased activity of APX and increased contents of GSH and ASC. On the other hand, excessive iron is also harmful to plant. The free ionic form, due to its catalytic action in one-electron redox reactions, can catalyze the formation of ROS through the metal-dependent Haber-Weiss and Fenton reactions (Asada and Takahashi, 1987). Thus, the iron concentration must be tightly controlled by plants and iron homeostasis is essential for plants.

Nitric oxide (NO) is a bioactive free radical which plays important roles in many physiological processes in plants, such as growth, development, senescence and adaptive responses to multiple stresses (Leshem et al., 1998; Beligni and Lamattina, 1999, 2001; Zhao et al., 2004; Graziano and Lamattina, 2005). Under ROS-related toxicity NO may act as a chain breaker and thus limit the oxidative damage. In the present study, we examined iron-deficiency induced oxidative damage in maize leaves and the effect of NO on stress symptoms and the antioxidant system during iron deficiency.

Materials and methods

Plant Material and Growth Conditions

Seeds of maize (*Zea mays* L. cv Zhongdan-2) were obtained from the Seeds Centre of Gansu Province, China. Seeds were surface sterilized in 1.8% (v/v) sodium hypochlorite, rinsed several times in distilled water, and germinated on moistened filter paper for 4 days. Seedlings were cultured hydroponically in a continuously aerated nutrient solution containing 5.25 mM KNO_3, 7.75 mM $Ca(NO_3)_2$, 4.06 mM $MgSO_4$, 1.0 mM KH_2PO_4, 46 μM H_3BO_4, 9.18 μM $MnSO_4$, 5.4 μM $ZnSO_4$, 9.0 μM $CuSO_4$, and 2.0 μM Na_2MoO_4 (Graziano et al., 2002). Iron was supplied as Fe(III)-EDTA in different concentrations ranging from 0 to 500 μM. The nutrient solution was adjusted to pH 5.5 and renewed once a week. Plants were grown in a growth chamber at a 60% relative humidity, 200 μmol photons $m^{-2}\,s^{-1}$ of light intensity and a 14 h/10 h (26 °C/22 °C) day/night regime. NO-treatment was performed by dissolving 100 μM sodium nitroprusside (SNP), an NO donor, in the nutrient solution once a week (+SNP). Plants grown without SNP were used as control (-SNP). Fully expanded fourth leaves of 20-day-old plants were harvested, weighed, frozen in liquid nitrogen, and stored at −80 °C for further analysis.

Nitric oxide determination

NO generation was estimated according to the procedure described by Murphy and Noack (1994) with some modifications. Fresh leaves (0.5 g) from maize seedlings were incubated with 100 U CAT and 100 U SOD for 5 min to remove endogenous ROS before the addition of 4.5 mL oxyhemoglobin (5 mM). After 2 min incubation, NO was measured spectrophotometrically by measuring the conversion of oxyhemoglobin to methemoglobin.

Determination of hydrogen peroxide (H_2O_2) and superoxide ($O_2^{-\bullet}$) contents

Hydrogen peroxide contents were determined by the modified method according to Shi et al. (2005). Leaf tissues were homogenized in an ice bath with 3% (w/v) trichloroacetic acid. The homogenate was